

# **<sup>13</sup>C-based Metabolic Flux Analysis of Environmental Microorganisms**

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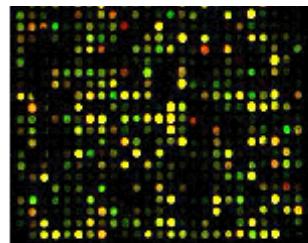
# Methods to Investigate Cellular Metabolism

DNA



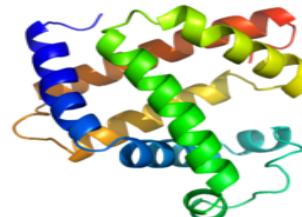
Genomics

mRNA



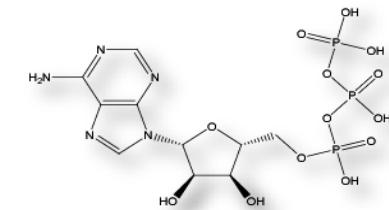
Transcriptomics

Enzyme



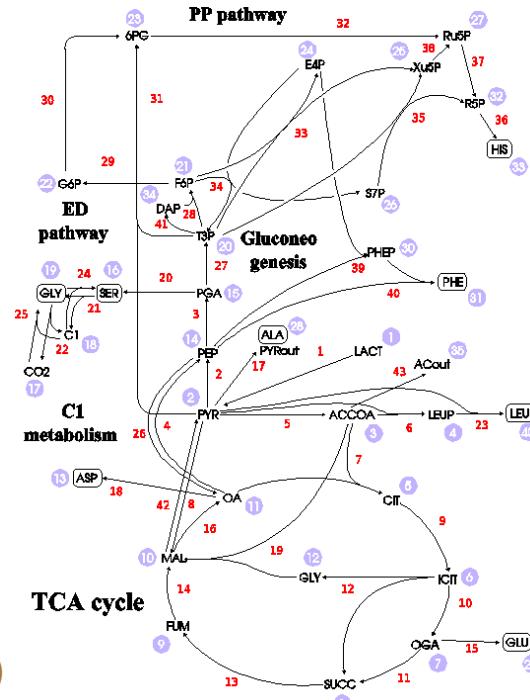
Proteomics

Metabolites



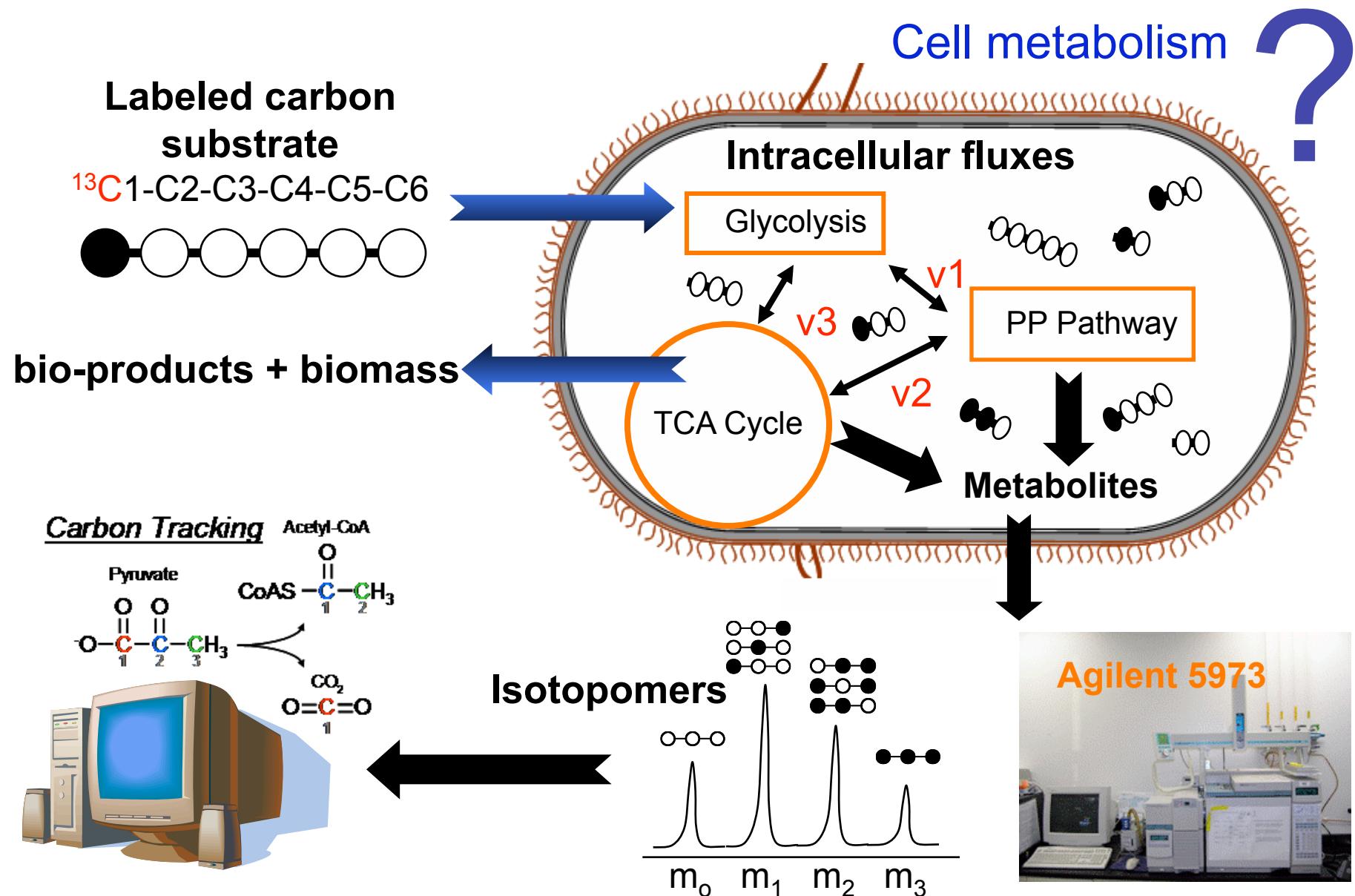
Metabolomics

Metabolic fluxes (*in vivo* reaction rates): functional output of combined omics



Tang et al., 2008, Mass Spectrometry Reviews (In press)

# $^{13}\text{C}$ Flux Analysis Protocol



# Experiments and Modeling

## Experiment



Chemostat Culture



Measure isotopic labeling in amino acids,  $M_{i,m}$



## Modeling

Build flux and isotopomer balance model

Select values for unknown fluxes  $v_n$

Compare  $M_{i,m}$  with  $M_{i,c}(v_n)$

$$\epsilon(v_n) = \sum_{i=1}^a \left( \frac{M_{i,m} - M_{i,c}(v_n)}{\delta_i} \right)^2$$

Predict isotopic labeling  $M_{i,c}(v_n)$

No

Minimal difference ?

$$\epsilon(v_n) \approx 0$$

Actual fluxes!

# Applications of $^{13}\text{C}$ Flux Analysis

- Investigate regulation of metabolism under various conditions.
- Identify unknown pathways and confirm gene functions.
- Reveal the bottleneck pathways for genetic manipulation.

## Environmental Microorganisms



Bioremediation (*Hanford site, WA*)



Biofuel production

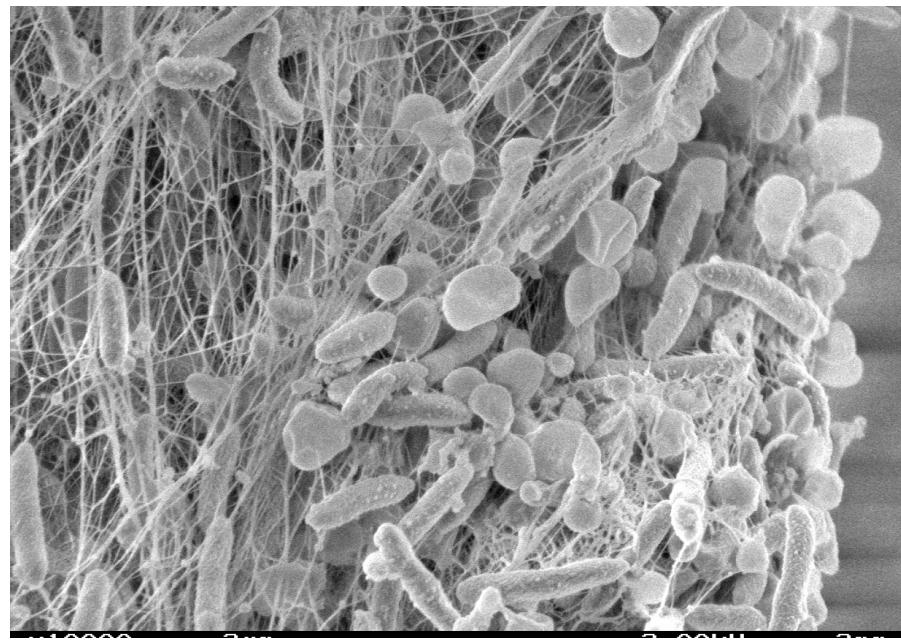
# 1. *Shewanella oneidensis* MR-1

Investigate central metabolism under oxygen conditions

## Versatile Metabolism

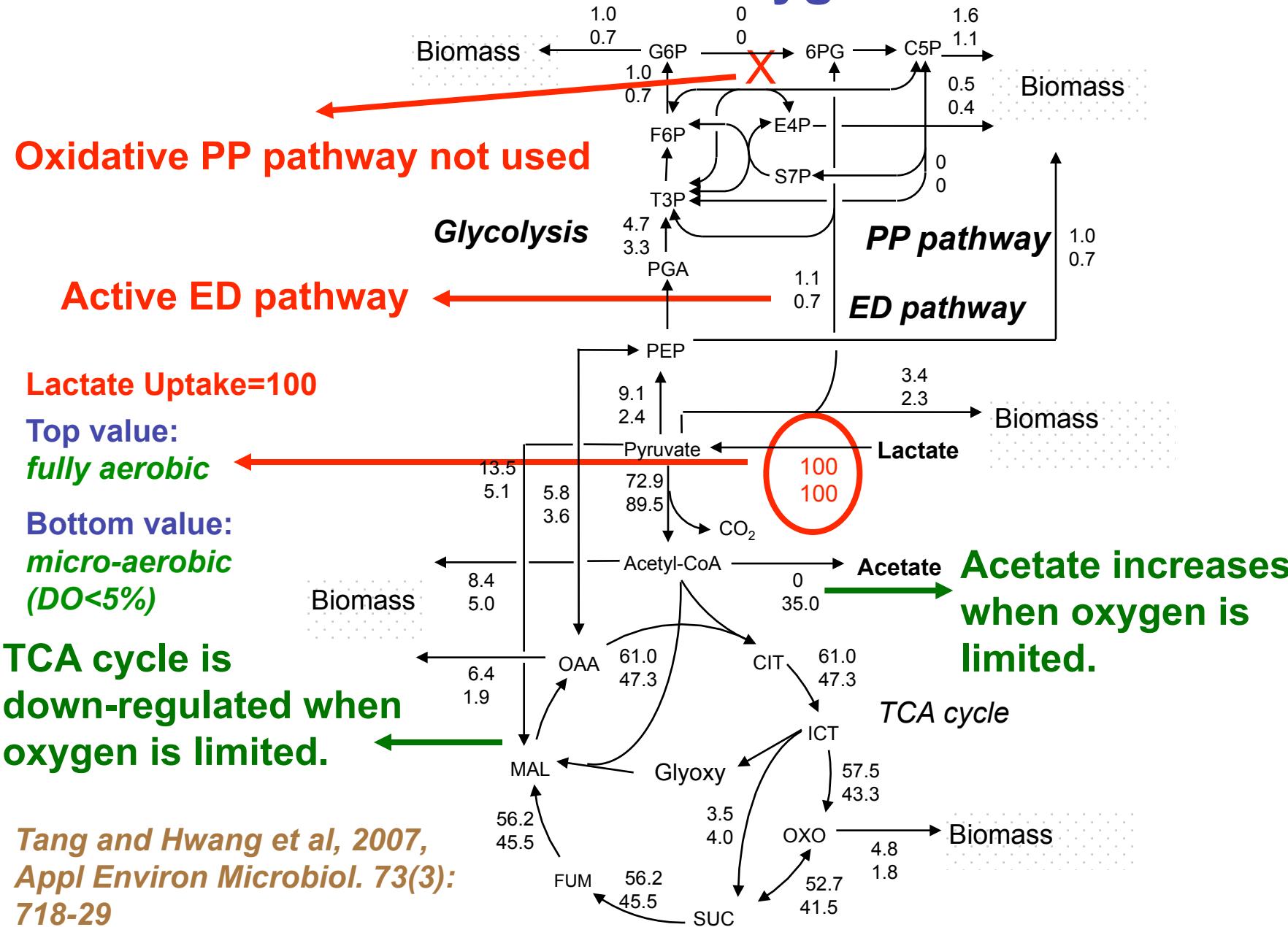
Electron acceptor:

$O_2$   
 $NO_3^-$   
Mn(IV)  
Fe(III)  
U(VI)  
Cr(VI)  
TMAO



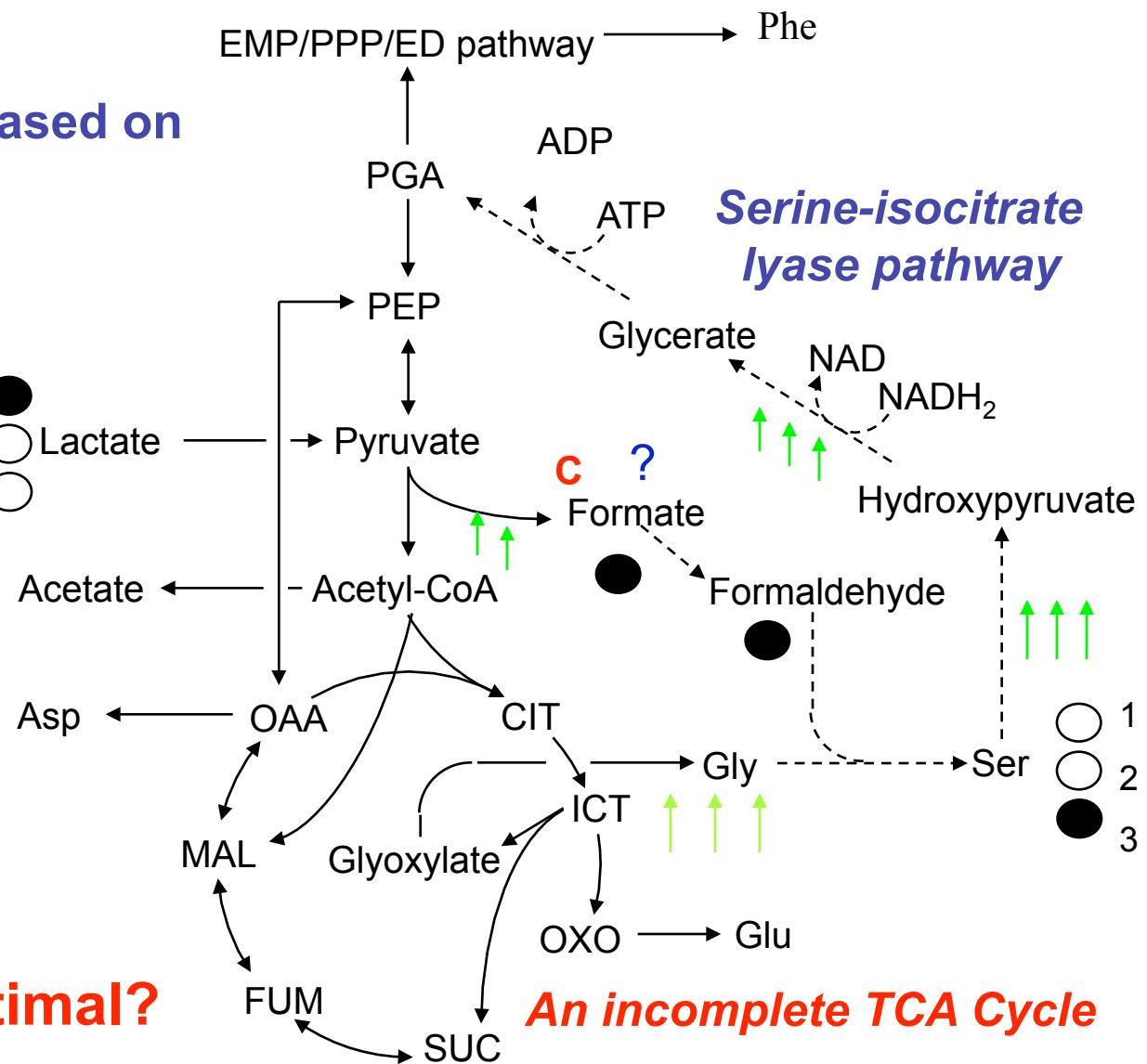
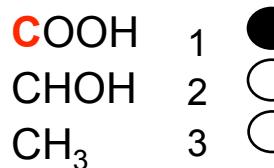
*Shewanella* “Nanowires” under oxygen limited conditions (Gorby *et al.*, Proc Natl Acad Sci 103(30):11358-63. Picture provided by Pacific Northwest National Lab)

# Fluxomes under Two Oxygen Conditions



# *Shewanella* Anaerobic Pathway

Anaerobic pathways based on enzyme chemistry and microarray data:



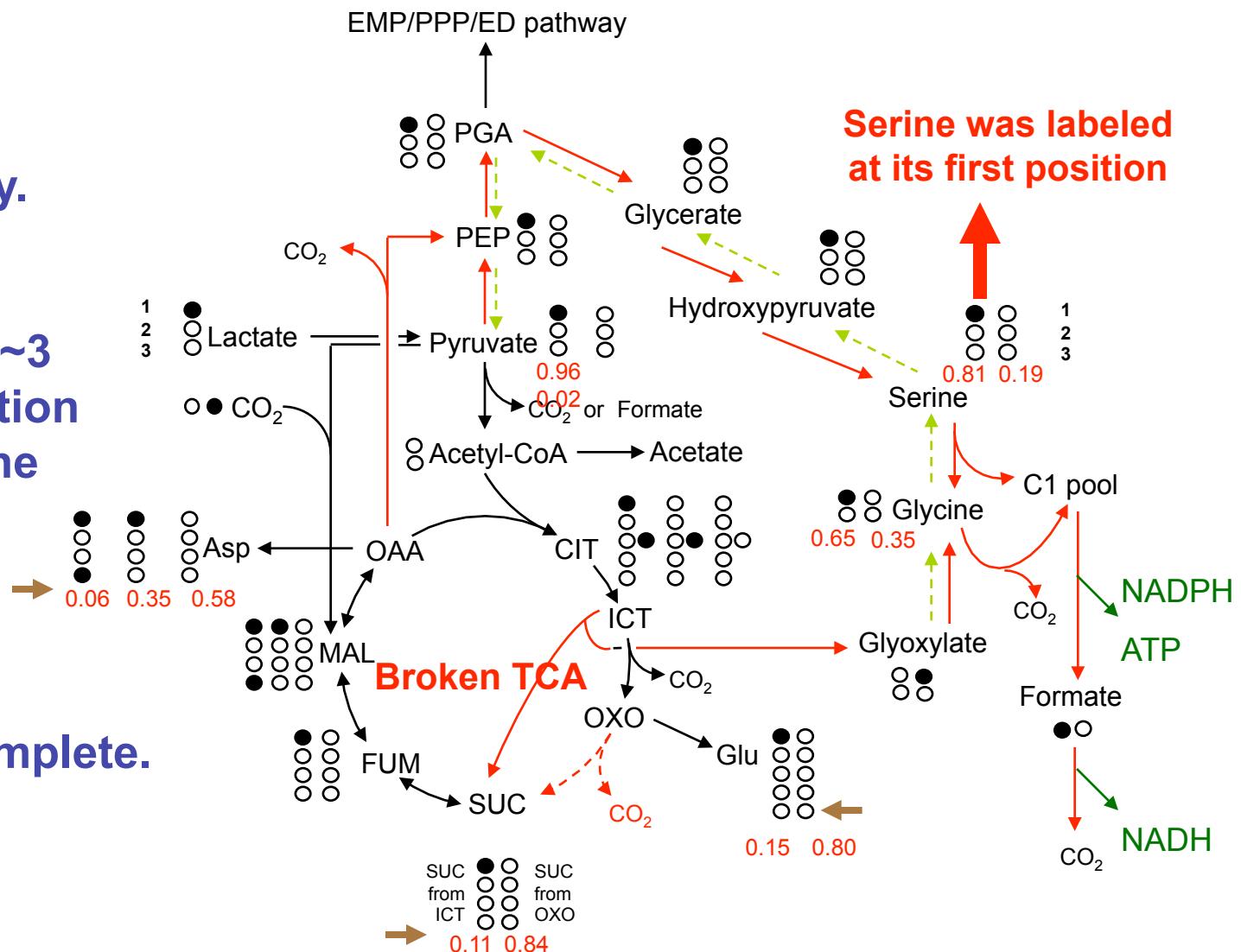
Is this pathway optimal?

An incomplete TCA Cycle

Scott and Nealson, 1994, J. Bact. 176(11):3408

# Anaerobic pathways based on isotopic data in light of enzyme chemistry and microarray studies

**1. Serine pathway provides energy.  
(Anaerobic biomass yield improves by >2~3 times with addition of serine, glycine or glyoxylate!)**

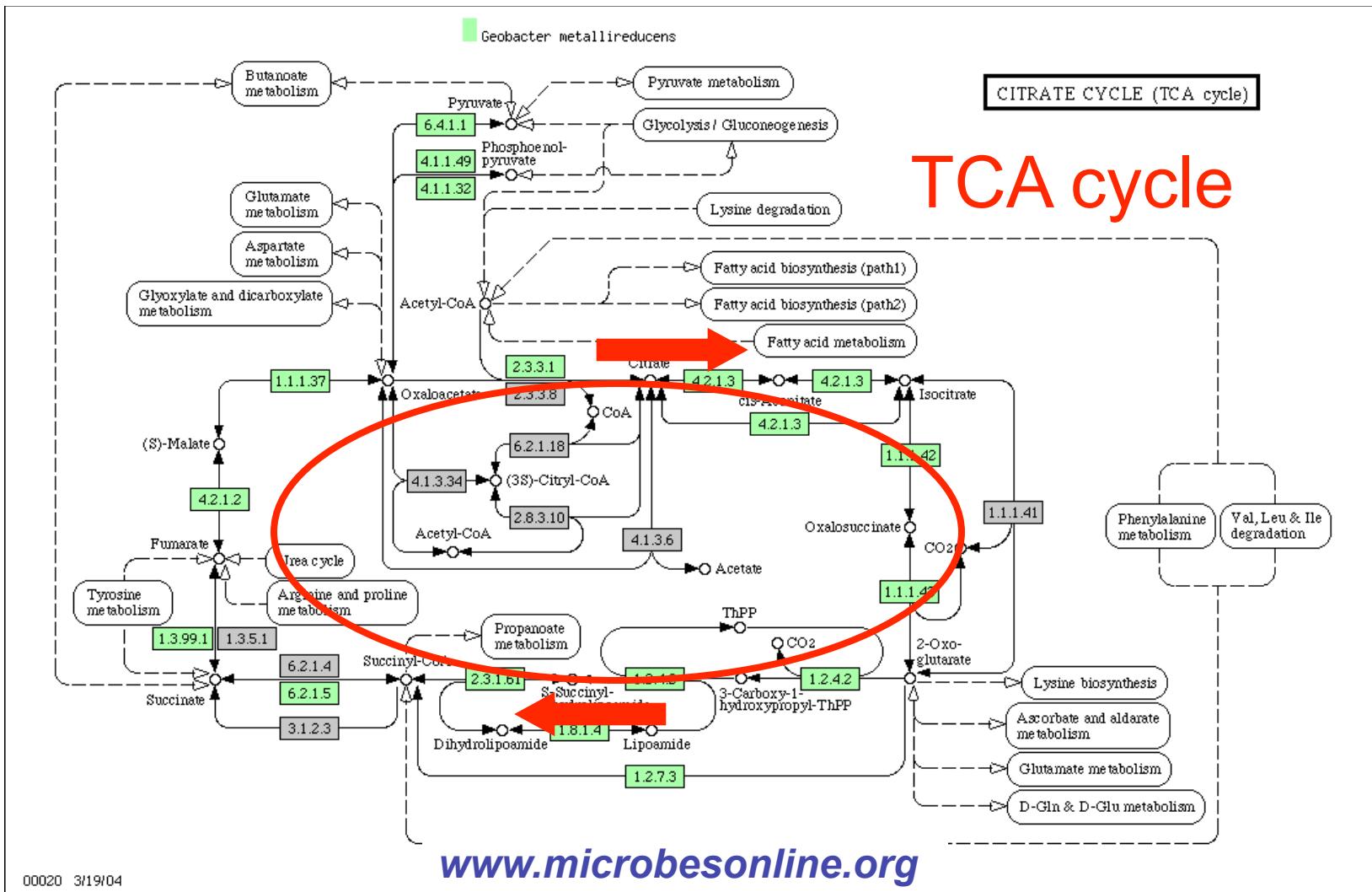


**2. TCA cycle is complete.**

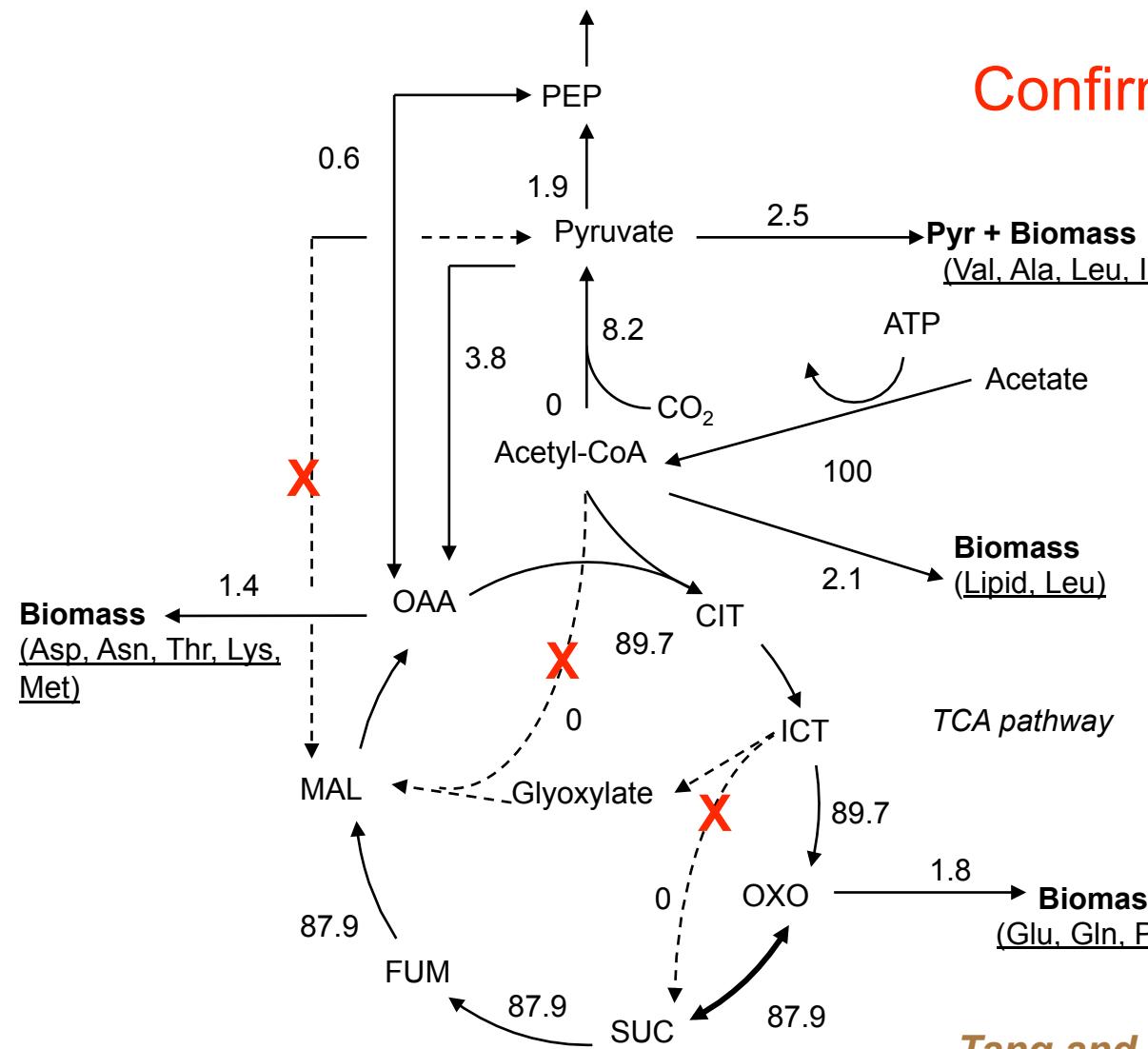
Tang et al, 2007, J. Bact. 189(3):894

## 2. *Geobacter metallireducens*

Confirm Gene Annotations



# Fluxes under Anaerobic Fe<sup>3+</sup> Reduction

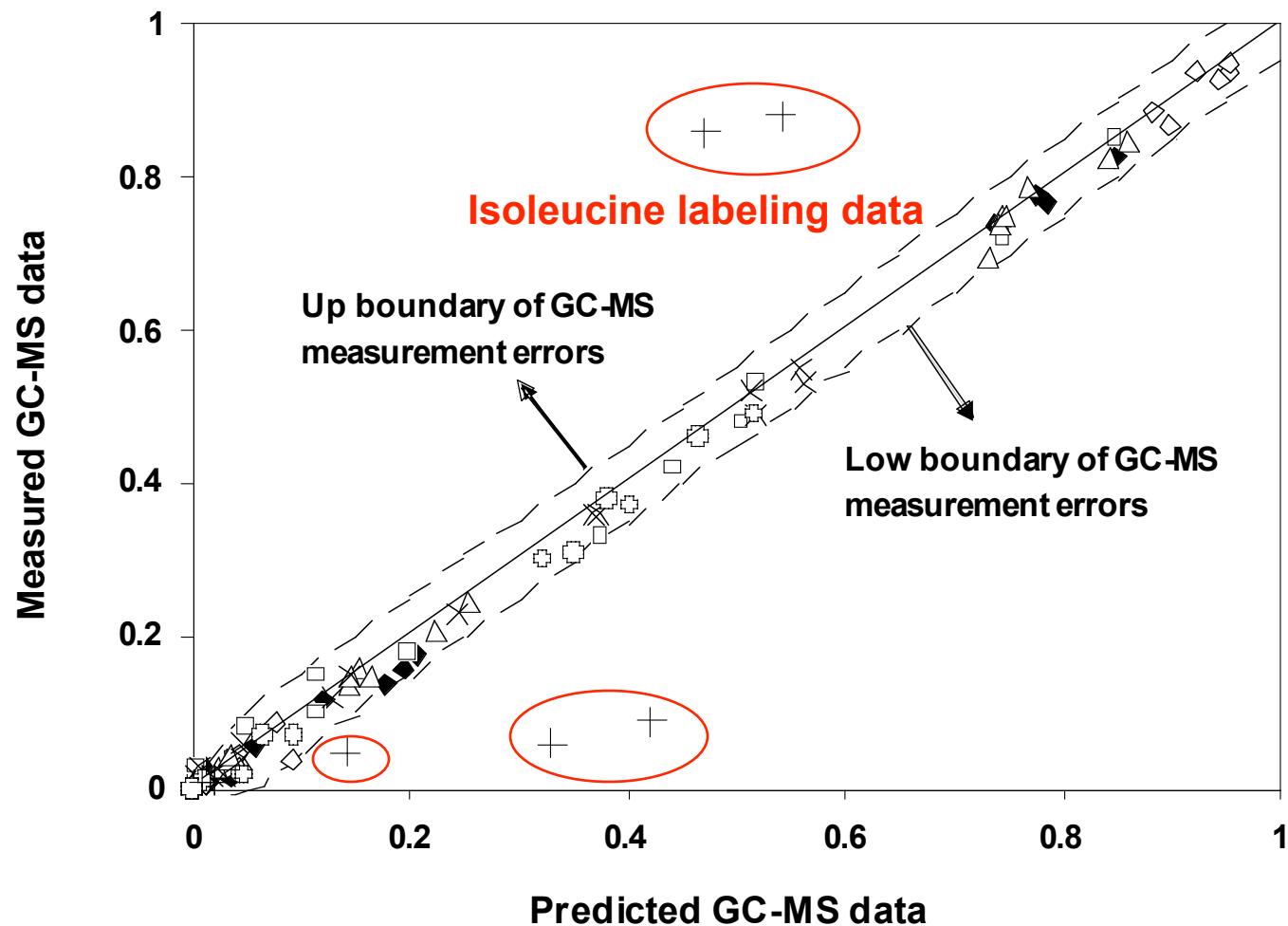


Confirm Gene Annotations

A complete  
TCA cycle in  
the anaerobic  
condition!

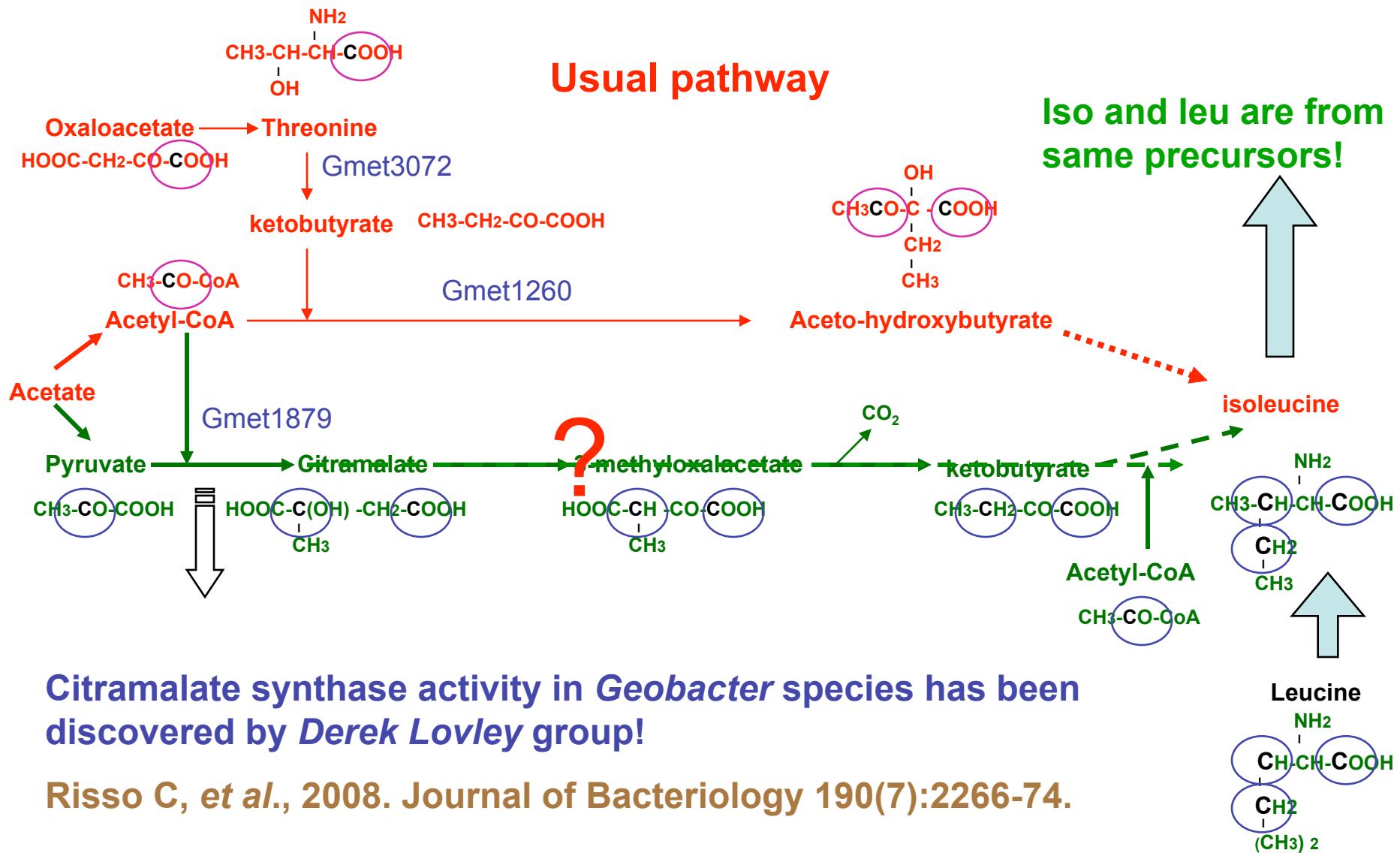
Tang and Chakraborty et al. 2007,  
AEM 73(12), 3859-64

# Finding An Unusual Isoleucine Pathway



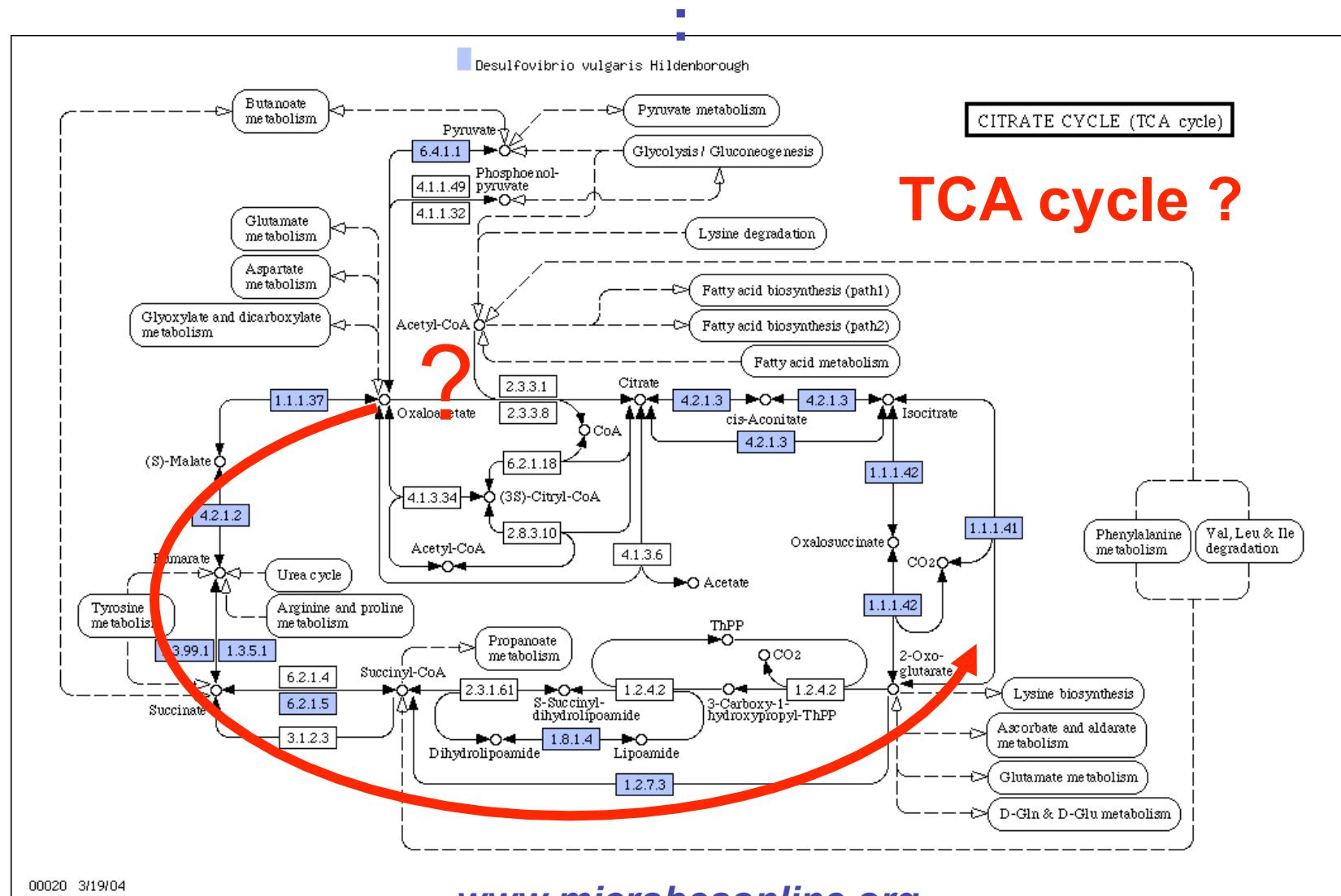
Tang and Chakraborty et al. 2007, AEM 73(12), 3859-64

# Unusual Isoleucine Pathway via Citramalate Synthase

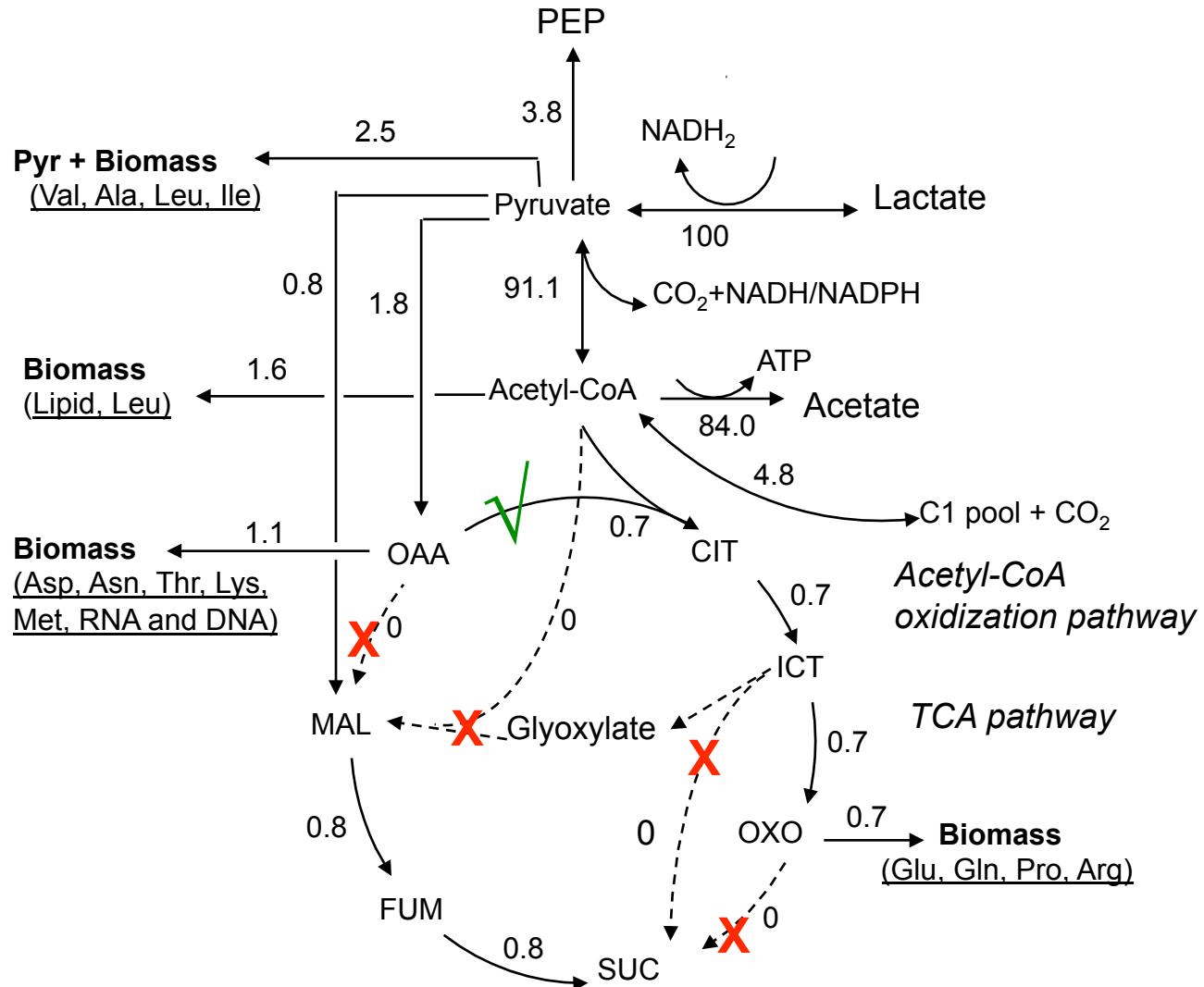


### 3. *Desulfovibrio vulgaris*

#### Finding New Enzyme

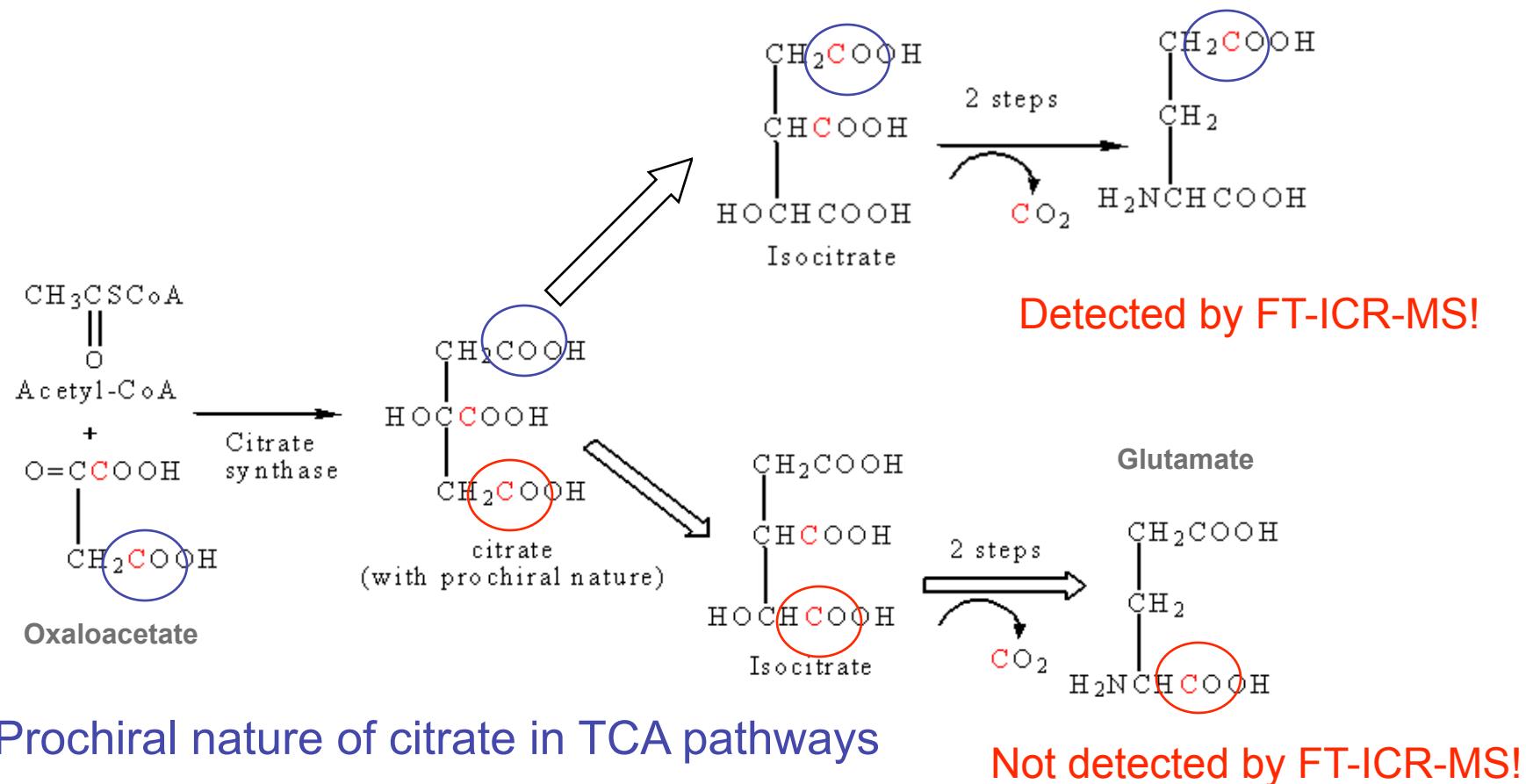


### 3. *Desulfovibrio vulgaris*: Investigate TCA Cycle



Tang, Pingitore and Mukhopadhyay et al., J. Bact. 189(3):940-9

# An Atypical Citrate Synthase (*R* type)



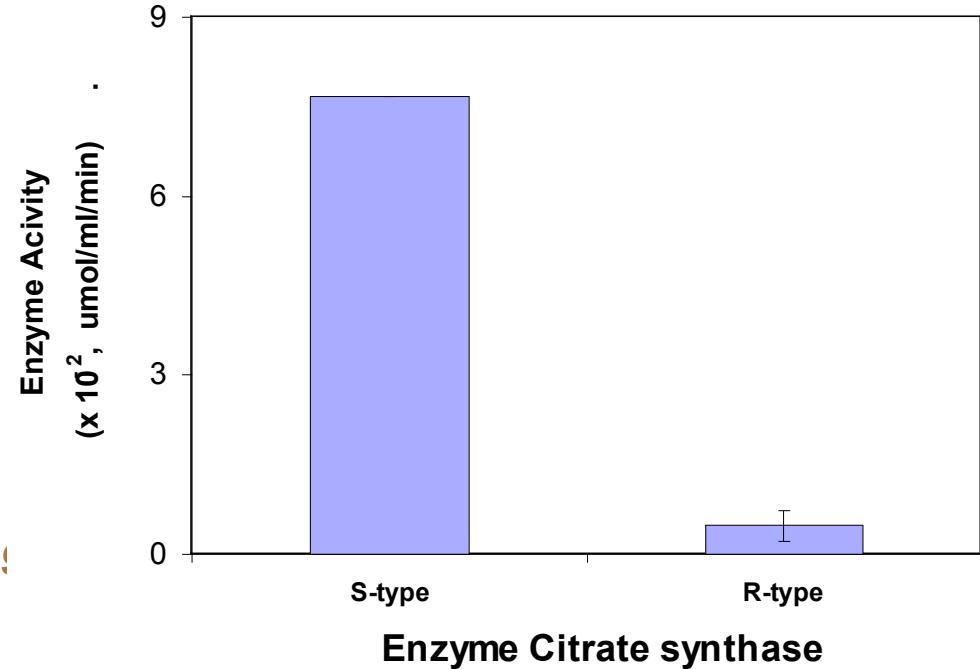
Pingitore and Tang et al. 2007, Anal Chem 79(6), 2483-90

# Recent Discovery on *R*-citrate Synthase

***R*-citrate synthase isolated from  
*Clostridium kluyveri***

**Phylogenetically related to  
homocitrate synthase and  
isopropylmalate synthase**

**Gottschalk et al., 2007, Journal of  
Bacteriology, vol. 189 No.11, pp429!**



**Investigate *R*-citrate synthase in *Desulfovibrio***

1. DVU0398 (~50% identical to the *C. kluyveri* citrate synthase) is the *R*-citrate synthase.
2. We successfully express this enzyme in *E.coli*.
3. *R*-citrate synthase is O<sub>2</sub>-sensitive.

**Price and Tang, et al., Paper in preparation**

# Journal publications

## Pubmed Data base (Nov 18, 2007)

Key words	Total Papers	Review	Earliest publication
<i>13C metabolic flux analysis:</i>	822	14	~1991
<i>Metabolic flux analysis:</i>	7870	382	~1960
DNA microarray:	26290	2972	~ 1994

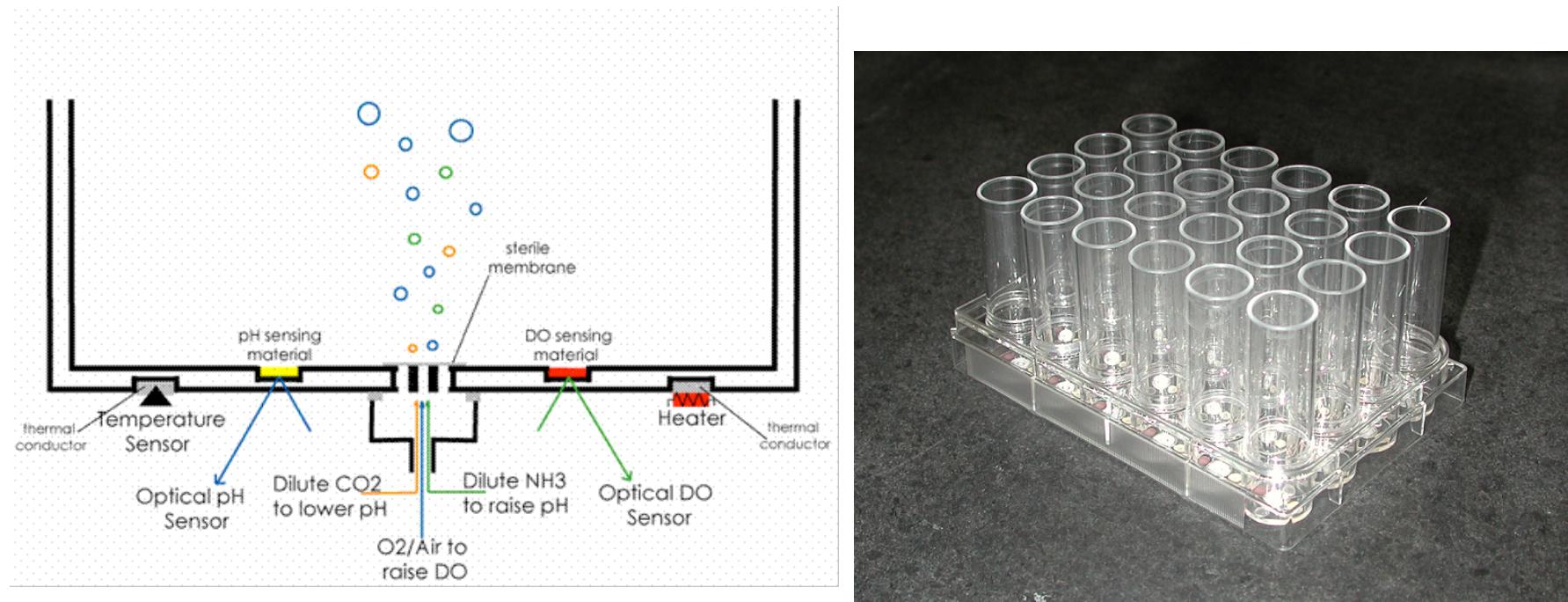
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**Improve  $^{13}\text{C}$  based flux analysis research?**

# Challenge 1: Steady State Culture

Bio-reactor fermentation: best control, but expensive.

Shaking flask: cheap but growth condition is not stable.



Mini-bioreactor: high throughput; low cost for labeled medium (~10mL); controlled growth conditions.

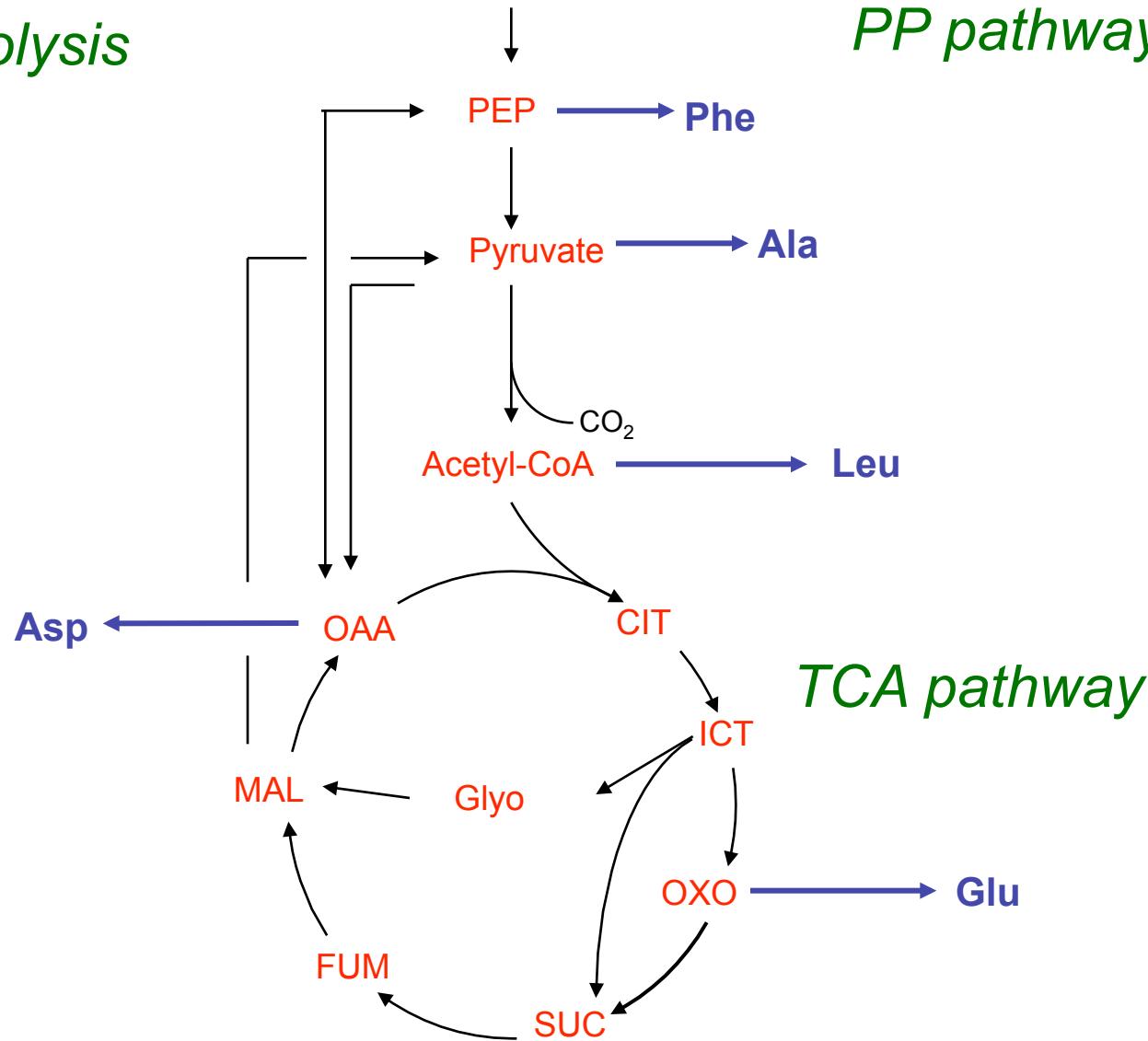
Tang and Laidlaw, et al. 2006 Biotechnol. Bioeng. 95(1):176-84.

# Challenge 2: Simple Metabolic Network

40~50 reactions

*Glycolysis*

*PP pathway*

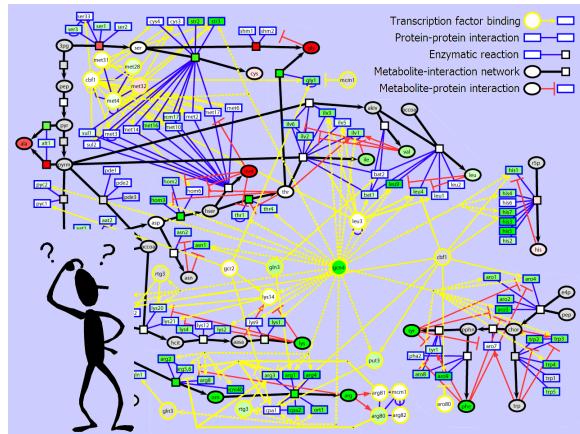


# Flux Analysis Beyond Central Metabolism

Free metabolites from *Mycobacterium*  
under non-replicating stage

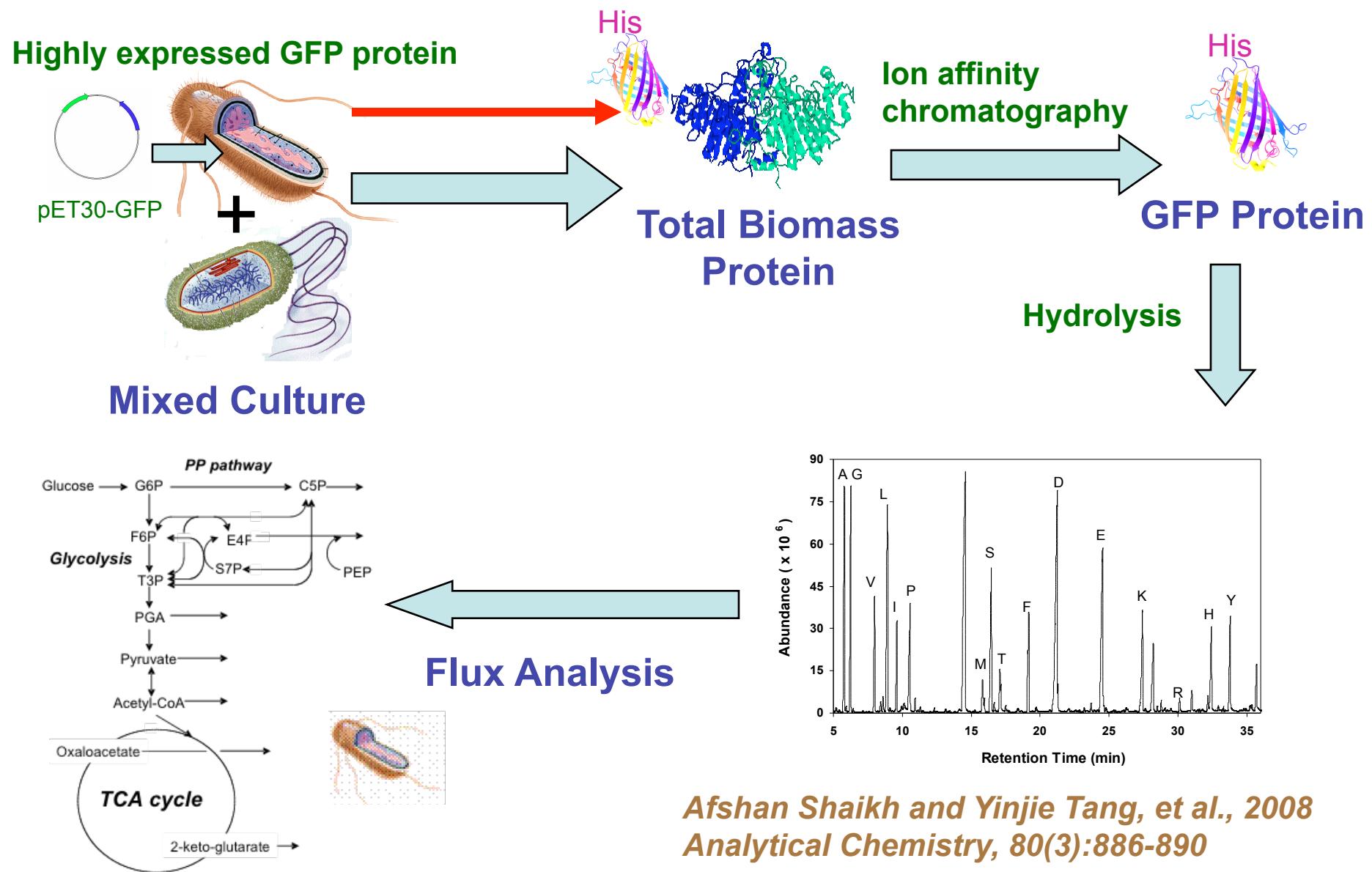
Metabolite	GC Retention Time	MW	Loss of - CH3	Loss of - COO-
alanine	5.6	233	218	116
arginine				
asparagine	12.0	348	333	231
aspartate	11.0	349	334	232
citrate	12.6	480	465	363
cysteine	11.3	337	322	220
fumarate	8.9	260	245	143
glutamate	11.7	363	348	246
glucose	13.3			
glycine	8.1	291	276	174
histidine				
isoleucine	7.9	275	260	158
a-ketoglutarate	11.8	377	362	260
lactate	5.1	234	219	117
leucine	7.6	275	260	158
malate	8.1	260	245	
methionine	11.1	293	278	176
oxaloacetate				
phenylalanine	11.8	309	294	192
proline	8.1	259	244	142
pyruvate	6.0	247	232	130
serine	8.7	321	306	204
succinate	8.3	262	247	
threonine	9.6	335	320	218
tryptophan	14.6	420	405	303

- CE-MS/ LC-MS
- FT-ICR-MS

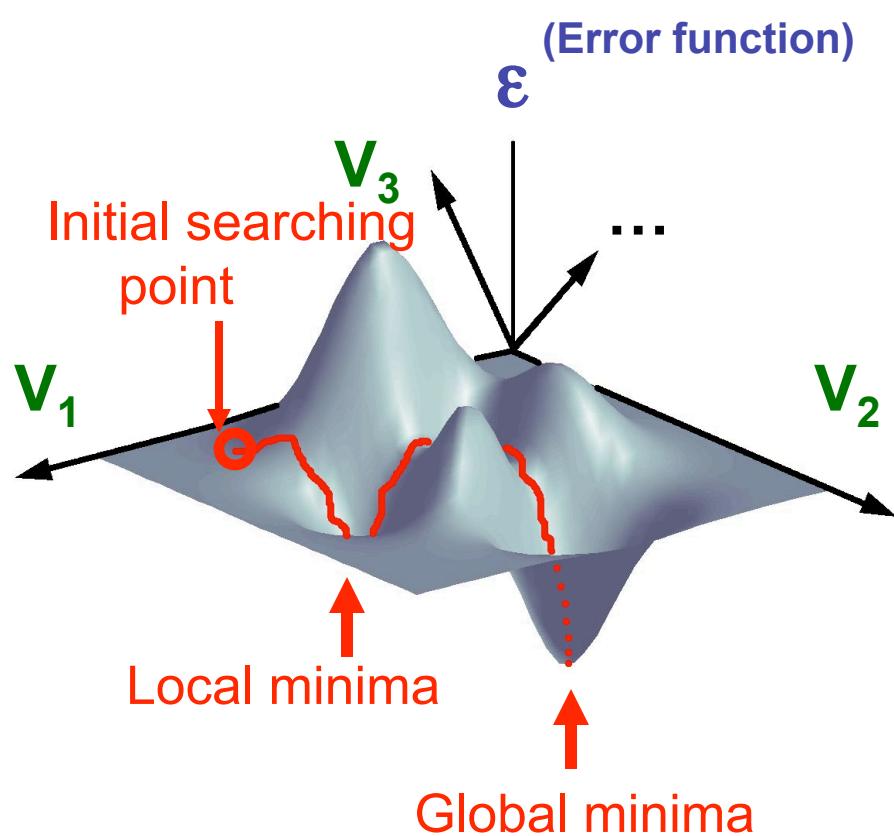


<sup>13</sup>C based flux analysis of genome scale metabolism

# Challenge 3: Mixed Culture Flux Analysis



# Challenge 4: Overcome Computational Bottlenecks



## Improving Searching Method

**Grid searching:** Search all possible flux combinations: very inefficient.

**Nonlinear solvers:** Nelder mead simplex ("fminsearch"): prone to fall in local minima.

**Simulated annealing:** not fooled by local minima.

**Genetic algorithms:** not fooled by local minima.

# Acknowledgement

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